comprising:

(a) cell extract supernatant depleted of activity of proteins that bind polyadenylate;

(b) other reagents; and

(c) directions for use of said kit.

a3 cancld

***Remarks***

Claims 1-55 are pending in the application. The foregoing amendments and following remarks are responsive to the Office Action mailed November 8, 1999.

The disclosure has been objected to for improperly referring to panels in the Brief Description of the Drawings. By the foregoing amendment to the Specification, this has been corrected. Withdrawal of the objection is requested.

The sequence rules have be complied with by the enclosed paper and disk copy of the sequences listed on page 63, as well as a statement that the contents are the same and no new matter is introduced thereby.

Applicants in amending claim 6 have included the term "uninfected" as another selection of the nature of the cell extract. Support for an uninfected cell extract may be found on page 5, lines 4-8, in which it is pointed out that the cell extract *may* be prepared from cells comprising foreign nucleic acid , such as those that are infected (emphasis added). Thus, the cells from which the extract is prepared may not necessarily be infected. Applicants have included the term to emphasize that one of the embodiments comprises uninfected cells or tissues.

***Claim Rejections - 35 USC § 112, Second Paragraph***

Claims 2, 7, 9-13, 15, 21-32, and 48-52 have been rejected under 35 U.S.C. § 112,

second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicants will address each specific point below and Applicant's amendment thereto and/or assertion with support that the term is definite.

In claim 2, "RNA" has been added to define the "C-rich element regulated turnover" to clarify this issue.

In claim 7: confusion over the term "partially purified" has been corrected by using in its place the phrase "a supernatant from a 100,000 x g, 1 hour centrifugation.," as supported on page 22, lines 1-2.

In claim 9, section (c), "bind" has been changed to "binds" to correct grammar. Moreover, a period has been added to the end of the claim.

In claim 10, line 2, "an" before the word "material" has been changed to "a.".

In claim 12: "consisting" has been added to correct the Markush group syntax.

In claim 15, line 2, the word "is" has been deleted to clarify the meaning of the claim.

In claim 21, section (A): "providine" has been corrected to "providing."

In claim 26, line 2, "is" has been deleted to correct the meaning of the claim.

In claim 27, dependency has been changed to claim 25; "monitoring." has been changed to "determining" to provide correct antecedent basis from claim 21, part (c); and in line 3, "sequence" has been added to "labeled target RNA" to provide correct antecedent basis to claim 25.

In claim 48, the dependency has been corrected to (method) claim 21.

In claim 51, section (B): "protein" has been changed to recite "endogenous molecule" to provide proper antecedent basis.

In claim 55, line 1, "of" has been inserted after "degradation" to correct the grammar.

In light of the foregoing amendments to the claims and the accompanying explanations, withdrawal of all of the rejections under 35 U.S.C. § 112, second paragraph rejections is courteously requested.

***Claim Rejections - 35 U.S.C. § 102***

Claims 1-2, 8-10, 12-15, 21, 24-30 and 51-52 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Bernstein. The Examiner asserts that Bernstein anticipates the claimed invention because, as in the claimed invention, the mRNA decay system taught by Bernstein comprises cell extract and target RNA wherein the target RNA is polyadenylated (claims 1-2). Bernstein depleted the cell extract of the activity of poly(A)-binding protein (claim 8) by various methods which included the addition of competitor RNA and passing the extract through a poly(A)-Sepharose column; in one experiment, Bernstein adds antibody to the protein to prevent interaction of the protein with poly(A) (claims 9-11); the mRNA used was labeled with 32P to analyze degradation (claims 14-15); Bernstein adds the endogenous poly(A) binding protein exogenously to the system and observes the effect on degradation of the mRNA (claims 21, 24-27 and 51-52); this resulted in increased stability of the RNA (claim 28). The Examiner goes on to point out that addition of antibody to the poly(A) binding protein prevented the stabilization effect of the protein through modulation of activity of the poly(A) element and therefore resulted in decreased stability of the RNA (claim 29); and further, to test the effect on mRNA stability by exogenously added PABP, Bernstein first introduced competitor poly(A) RNA ("RNA stability modifier"), then introduced exogenous PABP ("agent"), then determined the extent of turnover of the target RNA and identified the exogenous PABP ("agent") as capable of modulating the stability of the RNA in the presence of the previously introduced competitor poly(A) RNA (claims 33, 36-40, and 43).

Applicants respectfully traverse the rejection. By way of the foregoing amendment to

claim 1, the cell extract recited in the claim is now directed to that isolated from eukaryotic cells or tissues as was present in former claim 3, now cancelled. Moreover, the term "turnover" in claim 1 has been changed to "deadenylation and degradation" as set forth on page 15, line 22-23 of the Specification: "[t]urnover comprises deadenylation and degradation." As a result of the claim amendments, the instant claims no longer read on Bernstein because Bernstein used a ribosomal salt wash, an isolated component of a cell extract, obtained from eukaryotic cells, in the mRNA decay system, and furthermore, the Bernstein decay system found only RNA degradation, but not deadenylation. In contrast to the Bernstein system in which the use of an isolated ribosomal salt wash resulted in RNA degradation without deadenylation, which is not in-vivo turnover, the instant inventors used a cell extract which did not comprise an isolated cell fraction. Other than optionally removing nuclei and nuclear contents (see page 21, line 23-25), or *excluding* ribosomes by centrifugation at 100,000 x g (page 22, lines 1-2), Applicants' cell extract permitted the desired recapitulation. Applicants believe that the term "cell extract" is descriptive in that it is not an isolated subcellular component. Because deadenylation is well known to be a necessary prerequisite for in-vivo RNA turnover, and Bernstein does not demonstrate deadenylation, the Bernstein reference does not recapitulate regulated RNA turnover. Applicants therefore submit that the claims no longer read on the Bernstein reference, and withdrawal of the rejection is believed to be in order and respectfully requested.

Claims 1, 3-7, 12, 14, 16-17, 21-25, 28 and 55 have been rejected under 35 U.S.C. 102(b) as being anticipated by Krikorian. The Examiner asserts that the in vitro mRNA stability testing system of Krikorian anticipates the claims because, as claimed in the present invention, the system of Krikorian comprises a cell extract and target RNA (claim 1), the RNA is messenger RNA (claim 12 and 14) and the cell extract is isolated from lysed

HeLa cells (claims 3 and 4), cells are transfected with foreign nucleic acid (claims 5 and 6) and cell extract is "partially" purified (claim 7). Furthermore, the Examiner goes on to assert that Krikorian tests the effects of the addition of ATP, GTP, creatine phosphate, or creatine phosphokinase (claim 17); the system as described by Krikorian entails the addition of agents into the mRNA stability testing system described above and determining the extent of turnover of the RNA sequence in question (claims 21 and 25) in the presence and in the absence of ATP, GTP, creating phosphate, or creatine phosphokinase (claims 22-23 and 55). The Examiner continues to point out that one of the agents examined in Krikorian's system was proteinase K which modified the stability of mRNA molecule (claim 24) in particular, resulted in increased stability of the mRNA molecule (claim 26).

Applicants respectfully traverse the aforementioned rejection. Applicants submit first that the Krikorian system did not utilize any source of exogenous RNA, in contrast to the instantly claimed invention in which the RNA studied is from an exogenous source. Furthermore, Krikorian do not demonstrate deadenylation of mRNA (see figure 2, where shortened [deadenylated] RNA would have been evident if the system had recapitulated RNA turnover by deadenylation followed by degradation). Moreover, the Krikorian observations on RNA turnover were made only with HSV-infected HeLa cells as the source of extract, not uninfected cells. In light of these clear differences between the instant claims and the Krikorian reference, Applicants believe that the instant claims are not anticipated by Krikorian, and withdrawal of the rejection is courteously requested.

***Claim Rejections - 35 USC § 103***

Claims 31-32 and 44-45 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Bernstein in view of Chen and in further view of any one of Zhang, Myer, Nakagawa, Levine, Nagy, Nakamaki, or Liu. The Examiner asserts that Bernstein teaches an

in vitro system and method for evaluating mRNA stability: poly(A) binding proteins are added to a system of mRNA and cell extract and the resulting effects on mRNA stability are observed. The Examiner acknowledges that Bernstein does not demonstrate the role of other RNA binding proteins in mRNA stability.

The Examiner points out that Chen describes inhibitors that inhibit mRNA decay: AU-A and hnRNP Al proteins were observed in the cytoplasm after treatment of cells with Actinomycin D or DRB. The Examiner goes on to indicate that both of these proteins had been shown to bind to ARES in studies, Chen states that several other ARE-binding proteins were also found in the cytoplasm and nucleus and further explains the disruption of functional ARE-protein complex formation leading to mRNA stabilization. The Examiner acknowledges that Chen does not describe other RNA binding proteins affecting mRNA stability.

The Examiner points out that Zhang teaches the binding of AUF1, an AU-rich element RNA-binding protein to and AU-rich element in the 3' untranslated region of mRNAs and provides data on the role of this protein in mediating ARE-directed mRNA degradation; Myer teaches the binding of HuR to mRNA as a protein in AUUUA-mediated mRNA decay and that HuR is shown to bind selectivity to the AU-rich element in mRNA and play a role in the regulation of mRNA degradation; Nakagawa teaches AUH, a gene that codes a protein that binds specifically to AU-rich transcripts; Levine teaches Hel-N1, an RNA-binding protein with 3' UTR mRNA specificity and requiring a sequence containing AUUUG, AUUUA, and GUUUUU; Nagy teaches glyceraldehyde-3-phosphate dehydrogenase, a protein that selectively binds AU-rich RNA and suggests its role in the regulation of ARE-dependent mRNA turnover; Nakamaki teaches hnRNP C and AUF1 as AU-rich element binding proteins and determines that AU-binding factors, including hnRNP

C and AUF1, may be involved in rapid degradation of mRNA transcripts; and Liu teaches Hu antigens coded by HUD, HuC and Hel-N1 genes are homologues of Elav proteins and bind to AU-rich elements of mRNAs that regulate cell proliferation. It is the Examiner's opinion that one of ordinary skill in the art would have been motivated to evaluate the effect of additional ARE RNA binding proteins in the system of evaluating mRNA deadenylation and degradation because the system had been used earlier for evaluating one such protein by Bernstein with the conclusion at that time that ARE RNA binding proteins affected mRNA stability. The Examiner goes on further that Chen described a potential mechanism for altering mRNA stability by proteins that bind to the ARE of mRNA and cites two proteins in particular (AU-A and hnRNP A1) that have affinities for the ARE of mRNA and alter mRNA stability; the mRNA instability system and method of Bernstein was used for analyzing the effect of binding proteins and analysis on PABP (poly(A)binding proteins) was performed. The Examiner also points out that Zhang, Myer, Nakagawa, Levine, Nagy, Nakamaki, and Liu each demonstrate the binding patterns of various RNA binding proteins (AU-A, hnRNP Al, AUF1, HuR, AUH, Hel-N1, glyceraldehyde-3-phosphate dehydrogenase, hnRNP C, HUD, and HuC) and their roles in binding to ARE of mRNA and affecting mRNA stability. The Examiner concludes that it would have been prima facie obvious to one or ordinary skill in the art to utilize the system and method outlined by Bernstein wherein an RNA binding protein that binds to ARE in mRNA was analyzed as to its effects on mRNA stability and test other known RNA binding proteins that have the same function as the protein analyzed by Bernstein.

Applicants respectfully traverse the aforementioned rejection. By way of the foregoing amendment to claims 21 and 33, the goals of the claimed methods have been pointed out more clearly by the addition of the term "in-vivo" to "stability" to indicate that

the agent is intended to modulate in-vivo RNA turnover, i.e., deadenylation and degradation. Applicants point out (as also noted above) that the Bernstein reference never examined RNA deadenylation, a necessary prerequisite for in-vivo RNA degradation and thus a requirement for recapitulating RNA turnover. Therefore, no combination of references with Bernstein renders obvious methods for identifying agents that modulate the stability of a target RNA to turnover because in-vivo turnover involves deadenylation as a prerequisite for degradation, and the Bernstein reference does not recapitulate in-vivo RNA turnover nor appreciate the role of deadenylation therein. In light of the above and foregoing, withdrawal of the rejection is courteously solicited.

Claim 47 has been rejected under 35 U.S.C. § 103(a) as being unpatentable over Bernstein in view of Brewer and in further view Krikorian. The Examiner points out that Bernstein demonstrates a system and method for evaluating the effect of exogenously added agents (PABP) on the stability of poly(A) mRNAs, a system comprising target RNA and cell extract depleted of poly(A) binding protein is utilized wherein exogenous PABP is introduced into the system and the effect of PABP on the resulting degradation of mRNA is evaluated. The Examiner acknowledges that Bernstein does not teach the monitoring of deadenylation and degradation of target RNA and does not explicitly teach the relationship between deadenylation and degradation of target mRNA. The Examiner points out that Brewer demonstrates a system and method for monitoring deadenylation and degradation of target RNA and teaches that poly(A) shortening precedes degradation of mRNA with AU-rich sequences at the 3' end, but Brewer does not specifically teach the addition of nucleotide triphosphate to the system of mRNA turnover. The Examiner points out that Krikorian demonstrates the use of an in vitro mRNA degradation system, the system comprising target mRNA and cell extract: in an experiment to determine whether virion host shutoff-induced in

vitro mRNA degradation was dependent upon the components of an energy- generating system, parallel in vitro degradation experiments were conducted in which half of the reactions contained all of the components of the standard reaction, including ATP, GTP, etc, and the other half did not contain these elements. The degradation of mRNA was then observed. It is the Examiner's opinion that one of ordinary skill in the art would have been motivated to utilize a system of evaluating mRNA decay in the presence of a nucleotide triphosphate, then introduce an agent to evaluate the ensuing effects on the deadenylation and degradation on the target RNA because Brewer taught that polyadenylation of the mRNA precedes degradation of the RNA and Bernstein had earlier described and utilized a system of evaluating exogenously introduced agents introduced into the system and observing the resulting effect on mRNA stability by monitoring the degradation of the mRNA.

The Examiner goes on to assert that Krikorian had also demonstrated the use of such a system to evaluate mRNA degradation in the presence of ATP, GTP, etc., and that Bernstein further teaches that polyadenylation is an ATP-dependent process and indicates the presence of other ATP-dependent processes present in the procedure and Krikorian demonstrates the use of ATP, GTP, etc. in the performance of evaluation of mRNA turnover in his system. It is the Examiner's opinion that one of ordinary skill in the art, knowing the sequence of events in mRNA degradation, i.e., deadenylation preceding degradation, also knowing that ATP-dependent steps are present in the process as per the teachings of Bernstein, and following the teachings of Brewer in evaluating deadenylation and degradation of mRNA, would have utilized the system taught by Bernstein of evaluating mRNA deadenylation and degradation as well as first introducing a nucleotide triphosphate (ATP) as an energy source of required energy for ATP-dependent steps in the process since Bernstein had taught of the need for ATP and Krikorian had demonstrated such a use of ATP in an in vitro system to evaluate

mRNA turnover.

Applicants respectfully traverse the aforementioned rejection. Applicants submit that because no previous reference demonstrated (i.e., recapitulated) in-vivo RNA turnover (deadenylation and degradation), that the method of claim 47 which uses the method of claim 1 for identifying an agent capable of modulating the deadenylation and degradation of target RNA, would not be detectable in these references nor obvious from their combination. In light of the above, withdrawal of the rejection is requested.

Claim 53 and 54 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Bernstein. The Examiner notes that the claims are drawn to a kit comprising cell extract depleted of activity of proteins that bind poly(A) and other reagents. The Examiner indicates that Bernstein utilized a system in which poly(A) mRNA stability was evaluated: cell extract was depleted of poly(A) binding protein since RNA binding proteins that bind to ARE of mRNA was known to affect the stability of the mRNAs. It is the Examiner's opinion that one of ordinary skill in the art would have been motivated to package the cell extract and reagents of Bernstein into a kit because it was well-known and common knowledge in the art to package together reagents into a kit to facilitate practice of methods requiring said agents.

Applicants respectfully traverse the rejection. By way of the foregoing amendment to claim 53, the nature of the cell extract as described in the Specification as a cell extract supernatant as defined on page 54, lines 10-15. As noted on page 21, line 25 to page 22, line 3, the cell extract comprises a centrifugation supernatant. As Bernstein used a ribosomal salt wash preparation (from the collected pellet of centrifugation) for the determination of RNA degradation (in contrast to deadenylation and degradation), and the instant inventors use the supernatant of such a preparation, Applicants submit that the instant kit is not obvious over Bernstein. Withdrawal of the rejection is respectfully requested.

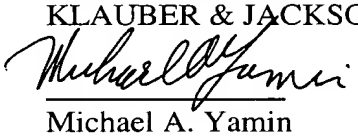
*Fees*

No fees are believed to be necessitated by the instant response. However, should this be in error, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment, or to credit any overpayments.

## CONCLUSION

Applicants respectfully request entry of the foregoing amendments and remarks in the file history of the instant Application. The Claims as amended are believed to be in condition for allowance, and reconsideration and withdrawal of all of the outstanding rejections is therefore believed in order. Early and favorable action on the claims is earnestly solicited.

Respectfully submitted,
KLAUBER & JACKSON



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